THE EFFECT OF TRACE MINERALS ON CELLULOSE DIGESTION AS STUDIED IN THE ARTIFICIAL RUMEN

By

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INTRODUCTION

The feeds normally consumed by cattle, sheep and other ruminants contain relatively large amounts of celluloses and hemi-celluloses. Utilization of feeds of this nature by ruminants is possible because of the activity of numerous microorganisms living in the rumen of the host animal. The animal is dependent upon the microorganisms for the breakdown of complex compounds into simple substances which can be utilized by the animal. Therefore, nutrition of cattle and sheep involves not only feeding of the animal but also the providing of conditions for optimal activity of the desirable types of microorganisms.

Not only are rumen microorganisms beneficial to the animal in breakdown of complex polysaccharides, but they are also responsible for the synthesis of proteins and amino acids, vitamins, especially those of the B complex group and in the formation of volatile fatty acids.

The recognition of the significant role played by microorganisms in the nutrition of ruminant animals has created the need for more critical study of rumen activity and of factors which affect this activity. Several _in vitro_ techniques have been developed and are useful in such studies. These methods have made possible the study of cellulose digestion and other studies of microbial activity under controlled conditions.

Among the dietary factors which are known to affect the activity of rumen microorganisms, particularly cellulose digestion, are the type and amount of carbohydrate, fat, nitrogenous compounds, the amount of vitamins, minerals, hormones, antibiotics, and certain unidentified factors.

Data on specific effects of different levels of trace minerals on cellulose breakdown and on the possibility of using the artificial rumen to improve rations high in fiber or cellulose for feeding monogastric animals are scant in the literature existing today.

This dissertation reports the results of investigations designed to determine: (1) the effect of different levels of copper, cobalt, iron, manganese, molybdenum and zinc on cellulose digestion, using an artificial rumen, (2) the effect of different types of diets, fed to cattle, upon the digestion of two purified celluloses in an artificial rumen, (3) the biological feeding value for rats and chicks of rations high in a purified cellulose after being fermented in an artificial rumen.

LITERATURE REVIEW

Techniques Used in Studying Rumen Microflora and Rumen Activity

For the study of rumen microorganisms, rumen activity and the many factors which affect the activity of the microflora, a number of different methods have been employed. Most of these techniques have included the usual methods of staining, isolation and cultivation, and microscopic examination. In recent years new *in vitro* and *in vivo* techniques have been developed.

Bacteriological techniques.--Differential counts of rumen microorganisms were used in early studies. Baker (2, 3) using the iodine staining technique, reported two types of organisms in the rumen, the iodophilic and the aniodophilic. He considered two types of iodophilic forms; the free population, consisting of microorganisms suspended in the ruminal and caecal liquids, and a fixed population which was attached to and acting upon the structural materials of the rumen contents. In later work it was pointed out that fixed forms were the most active and included the following morphological forms: curved rods and vibrios, very small coccoids, larger coccoids, giant coccoids, and giant ellipsoidal and other elongated forms (4). In studying the effect of diet on the rumen microflora, Bortree et al. (18, 19) also made use of iodophilic bacteria as indicator organisms.

The use of the number of microorganisms per unit of rumen content became popular, when a technique for counting the microorganisms present in the rumen was developed. The number of bacteria per gram of fresh rumen contents of healthy animals on practical farm rations has been shown to be in the neighborhood of 50 to 100 billion (48, 49). In later work, Gall et al. (50) reported that rumen contents from an animal deficient in cobalt contained 30.2 billion microorganisms per gram, while the contents from a normal animal contained 54.6 billion. This technique has been used extensively when comparing different types of diets fed to cattle or sheep.

Culture methods have been used by several workers to isolate rumen bacteria. As early as 1928, Bechdel et al. (5) isolated a microorganism, identified as Flavobacterium vitarumen, from a Holstein cow receiving a diet highly deficient in the vitamin B complex. This bacterium was grown on plain nutrient agar, free of vitamin B complex and when fed to rats as dried bacterial cells, it was found to be highly potent in the vitamin B complex. When the Holstein cow was changed to a diet containing B complex vitamins, this organism disappeared and has not been isolated since from rumen contents.

Many pure cultures of other species have been isolated from the rumen since the report by Bechdel et al. (5). Most of these have been cellulose decomposing bacteria. Twenty type cultures of rumen microorganisms have been isolated and described by Gall and Huhtanen (47). Bryant (21) has isolated 51 different kinds of bacteria from 102 cultures of rumen microorganisms. Isolation of an anaerobic, propionic acid producing coccus from the rumen of sheep has also been reported (69). A description of 33 types of rumen microorganisms of sheep with respect to shape, size, and gram and iodine staining, has been made by Moir and Masson (88). In addition, some observations on motility, formation of spores and other relevant properties were recorded. The anaerobic forms which digest cellulose have been classified into five wide categories: actinomycetes, thermophilic sporeformers, non-sporeforming rods, cocci and mesophilic sporeformers (67).

Recently, Bryant and Burkey (22) made a detailed study of cultural methods for rumen bacteria, and isolated 896 strains, all of which were strict anaerobes except the facultative anaerobes of the genus Streptococcus. The morphological types cultured included cocci, rod-shaped organisms, spirilla and spirochetes. Isolation of five types of small, thin, curved, motile rods, all of which were active in digestion of fiber, has been reported by Huhtanen and Gall (65). Three larger, short, straight rods occurring in chains, which appeared to be important and were non-sporeforming obligate anaerobes producing butyric, acetic and lactic acids were also isolated.

Although these methods have provided valuable information on certain aspects of the rumen microflora and rumen activity, a complete study of the rumen population can not be made by standard bacteriological procedures. Some organisms require special techniques, and the many morphological types of organisms in the rumen make such studies much more complex. This has led to a search for the subsequent development of additional techniques, two of which are the use of the artificial rumen, and the use of fistulated animals.

In vitro techniques: artificial rumen.--The artificial rumen as an in vitro technique is a method of providing, as nearly as possible, conditions similar to those existing in the rumen. The optimal conditions as established and recognized by most workers are: temperature of 39 to 40° C., pH of 6.0 to 7.2 and anaerobic conditions using carbon dioxide or nitrogen gas. This method of studying rumen activity offers a quick and relatively inexpensive means of obtaining information.

As early as 1884 Tappeiner (cited by Burroughs and Hall, 26) incubated rumen contents from cattle in closed jars at room temperature, and observed that crude fiber decreased after a few days. In later work he incubated rumen contents *in vitro* at 37° C. and observed after 14 days of incubation that 23 per cent of the crude fiber disappeared. When chloroform was added before incubation, cellulose decomposition did not take place.

Wegner *et al*. (105) added urea to rumen contents and after incubation at 37° C. determined inorganic nitrogen measuring in this way the conversion to protein. English workers (91, 92) collected rumen ingesta, strained it through muslin and incubated the filtrate at 39° C. in a water bath for 2 to 4 hours under one atmosphere of air. This procedure was used to study the effects of pH, type of carbohydrate present, temperature, gaseous environment, and urea concentration on the activity of rumen organisms. Both of these methods were criticised for the lack of movement or diffusion of the end products as in the live animal.

In 1948, Marston (82) reported the use of a technique which consisted of a closed jar containing the rumen contents. The jar was equipped with a mechanical stirrer for movement of the ingesta and a gas inlet and outlet system to maintain an anaerobic condition. McNaught (84) and McNaught *et al*. (85) modified this technique by centrifuging the strained rumen samples at 2,000 r.p.m. for five minutes. One per cent maltose and 0.05 per cent urea were added and the non-protein nitrogen determined. The sample was incubated for four hours at 39° C. and the non-protein nitrogen determinations were made again.

A rather complicated technique has been suggested by Louw (78) which consisted of a glass jar suspended in a water bath at a controlled temperature. A growth medium was added to the jar and suspended in the growth medium was a Visking casing through which the different by-products of fermentation could be dialyzed. The glass was provided with several inlets and outlets for the gassing, sampling and for the addition of buffers. The material was kept in constant motion by use of a stirrer. Huhtanen et al. (66) described a miniature artificial rumen technique for studying fiber digestion by rumen bacteria. The technique is similar to the artificial rumen devised by Louw (78) except that it is smaller, and the frequent neutralization is eliminated. An artificial rumen known as the permeable type, has also been described (46). It consists of a cellophane sack containing the inoculum and substrate immersed in the medium. The reaction takes place within the sack through which fermentation products may diffuse.

South African workers (62) have determined cellulose digestion by suspending cotton threads in incubated rumen fluid. The rate of disintegration is used as a measure of cellulolytic activity.

Burroughs et al. (23, 24, 25, 31, 32, 33) developed an artificial rumen which consisted of 500 ml. or 1,000 ml. size fermentation flasks incubated in a water bath at 40° C. Each flask was fitted with a three hole rubber stopper for the gassing with carbon dioxide and for the regulation of the pH of the contents. Fermentations were carried out continuously for 36-hour periods. The inoculum for the first fermentation was used directly from the rumen, but for the successive periods the inoculum consisted of half of the residue of the preceding fermentation. Cellulose digestion was determined chemically and gram stains were made at the beginning and end of each fermentation period.

Recently a method to improve the inoculation was developed by workers in Ohio (13, 14, 16, 17). Strained rumen liquid was centrifuged at high speeds and the sediment containing the microorganisms was suspended in an isotonic phosphate buffer solution containing 0.05 per cent cysteine. The culture medium was composed of cellulose, urea, minerals and glucose. The fermentations were carried out in liter low actinic Erlenmeyer flasks which were arranged in a water bath with the temperature controlled at 38 to 39° C. Flasks were gassed individually with carbon dioxide. The length of fermentation periods ranged from 24 to 48 hours.

At approximately the same time, Cheng _et al_. (34) developed a technique based on the use of a washed suspension of rumen microorganisms for the study of dellulose digestion _in vitro_. The strained rumen contents were centrifuged at high speeds and the sediment was washed several times with distilled water or a phosphate buffer. The final sediment was suspended in a complex nutrient solution and the pH adjusted to 7. This was followed by fermentation for 24 hours at 39° C. and under anaerobic conditions provided by carbon dioxide. The use of washed cell suspensions makes it possible to use an inoculum which is more nearly free of nutrients.

The _in vitro_ techniques have been criticised because a shift in the microflora may occur and the inflow of saliva and the movement of ingesta and end-products of fermentation _in vivo_ may not be duplicated. Although these criticisms may be valid, the use of the artificial rumen in many experiments has provided much useful information.

In vivo techniques.--The wide variety and number of _in vivo_ studies related to rumen digestion, which include feeding and digestion trials and experiments with fistulated animals, will not be reviewed here. Studies which have been particularly valuable have made use of the rumen fistula (40, 99). This device makes possible an easier collection of rumen contents from animals on a known dietary regime. The use of fistulated animals offers a means of studying the chemical, physical and microbial changes that occur within the rumen and also offers a means of forced feeding materials which otherwise are difficult to get into the rumen.

Effect of Various Dietary Factors Upon Cellulose Digestion

Results of the numerous experiments concerning rumen activity have demonstrated that many factors are capable of effecting a change in rumen activity. Among those which have been studied are dietary intakes of minerals, carbohydrates, fats, protein, non-protein nitrogen, antibiotics, hormones and certain unidentified factors.

The determination of these factors and their control which may be used to provide optimum conditions for proper rumen function have been the goals of research workers. Many of these factors which are known to alter microbial digestion in the rumen do not operate independently. As a rule all of these factors or conditions should be expected to have an interaction or interrelationships.

Effect of minerals on rumen activity.--At the present time the importance of the minerals in the nutrition of the ruminant is well known. Diseases have been reported from many areas as a result of deficiencies in minerals. Not only may a lack of an element cause a disease, but also a slight excess of certain metals in the diet of ruminants may lead to abnormalities. Perhaps some of the best-known examples are diseases caused by excesses of molybdenum and selenium. In some cases, elements that are essential may be toxic when present in excess; examples are copper and cobalt.

Of all the studies of the effect of minerals in rumen nutrition, only a few have been made relative to the effect of these elements on the rumen, and rumen bacteria.

It has been shown that alfalfa ash, water extracts of alfalfa, yeast, dried distiller's solubles, mineral mixtures, molasses ash, autoclaved water extract of cow manure, autoclaved rumen liquid all increased the digestion of cellulose (15, 16, 29, 32, 25, 72).

Tosic and Mitchell (103) considered that cobalt may be more necessary for the rumen microorganisms than for the ruminant. In cobalt deficient sheep the efficiency of digestion of nitrogen free extract and ether extract was reduced but digestion of fiber was not reduced (6). The number of bacteria present in rumen contents from normal sheep is almost double that of cobalt deficient sheep (50).

Klosterman et al. (72) found that an addition of a trace mineral mixture to a ration of soybean oil meal, corn and cob meal, and poor quality hay resulted in a marked increase in liveweight gains of cattle. The trace mineral mixture used in these studies consisted of cobalt, copper, manganese, iron, iodine, and sulfur. In further work (71), they confirmed the above results and found that molasses was of no apparent benefit when added to a ration which included trace minerals or good quality mixed hay.

Other workers have reported unfavorable results from feeding trace minerals. Plumlee _et al_. (93) found that twin beef calves receiving a ration largely composed of corn cobs ate less and grew at a slower rate when trace minerals were added. Tillman _et al_. (102) and Gossett and Riggs (52) reported that neither alfalfa ash nor a complete mineral mixture improved the digestibility of low quality prairie hay.

By using _in vitro_ techniques, it has been found that additions of molasses ash, phosphorus and iron stimulated cellulose digestion. The statement has also been made that factors other than iron, phosphorus, sodium, potassium, calcium, magnesium, chlorine and sulfur are involved in rumen physiology (32).

McNaught _et al_. (85), using the diminution of non protein nitrogen in rumen liquid when incubated _in vitro_ as an index of bacterial growth, without actually counting the bacteria, found that the microorganisms were able to tolerate 100 p.p.m. iron, 10 p.p.m. copper, somewhat less than 10 p.p.m. cobalt and between 100 and 1,000 p.p.m. molybdenum. Growth of the bacteria was definitely inhibited by 1,000 p.p.m. iron, 25 p.p.m. copper, 1,000 p.p.m. cobalt and 2,000 p.p.m. molybdenum. Salsbury *et al*. (95) reported that cellulose digestion by rumen microorganisms *in vitro* was slightly depressed by 12 p.p.m. of added cobalt and strongly depressed by 100 p.p.m.

Rubidium, cesium and lithium have not been found to be effective in stimulating greater cellulose digestion when ample potassium and sodium were present in the medium (63).

Effect of carbohydrates and fats on rumen activity.--It appears that the effect of carbohydrate and fat upon cellulose digestion is closely related to the other nutrients present. The amount of crude fiber digested in the rumen has been reported to range between 70 to 85 per cent (53, 54). This percentage is affected by all other ingredients in the ration (80).

Small amounts of readily available carbohydrates have been shown to increase the amount of cellulose digested (1, 33, 35). Hoflund *et al*. (62) found that the concentration of glucose for optimum cellulose digestion *in vitro* varied between 0.1 and 0.2 per cent. *In vivo* work (44) showed that the addition of 2 pounds of molasses to a grain and alfalfa hay ration fed to dairy cows, apparently increased the digestibility of crude protein, cellulose and crude fiber.

When large amounts of readily available carbohydrate are included in the ration, a decrease in crude fiber digestion is obtained (1, 12, 30, 44, 56, 62, 68, 77, 80). This is due to the preference of the microorganisms in the rumen for soluble carbohydrate (56). In vivo work (44) showed that additions of 4 pounds of molasses to a timothy-lespedeza hay mixture for dairy cows apparently decreased the digestibility of crude protein, cellulose and crude fiber.

Work involving the bacterial population as affected by carbohydrate has been reported by Bortree et al. (18). They observed that the addition of 3 pounds of sugar to a hay ration resulted in a 100 per cent increase in the bacterial population over that of hay alone. When starch was used at the same rate, only a slight increase in bacterial population was obtained.

Little work has been done on the effect of fats upon rumen microorganisms. Swift et al. (100) fed rations containing fat levels varying from 3 to 10 per cent. Corn oil replaced cerelose on a caloric basis. Utilization of protein and energy was similar in all trials reported. The addition of 10 mg. corn oil to a 25 ml. artificial rumen increased the breakdown of cellulose, while levels of 20 to 80 mg. resulted in a decrease (20). Willey et al. (108) reported that adding 5 per cent cottonseed oil to a practical fattening ration for steers resulted in a more efficient feed utilization. Indications that valeric and caproic acids and to a lesser extent iso-butyric and iso-valeric acids increased the rate of cellulose digestion and ammonia utilization *in vitro* has been shown by Bentley *et al*. (14, 16).

Effect of nitrogenous compounds on rumen activity.-- For a number of years the utilization of nitrogenous compounds by the ruminants has been of interest to the research worker not only from the point of view of protein synthesis but for their effect on other activities performed by the microflora in the rumen, particularly cellulose digestion.

One of the early reports of the use of non-protein nitrogen for ruminants was that published by Hart *et al*. (58) in 1939. Using urea and ammonium carbonate in a ration for dairy heifers, they obtained results which were interpreted to mean that nitrogen from these compounds was converted by the microorganisms into microbial protein. Since the time of this report, many other experiments have confirmed this conclusion (24, 105, 106, 107).

Several reports have appeared in the literature showing that cattle can use non-protein nitrogenous compounds among which are: ammoniated beet pulp, ammoniated cane molasses, ammoniated condensed distillers molasses solubles, urea, ammoniated industrial by-products and other ammoniated feedstuffs (38, 45, 73, 74, 79, 87, 101). Nitrogen of numerous organic and inorganic ammonium salts has also been shown, by in vitro work, to serve as sources of nitrogen for the rumen bacteria (10).

One of the conditions necessary for efficient utilization of non-protein nitrogenous compounds by the ruminant is the use of a low protein ration. If large amounts of natural protein are present in the ration, little conversion of non-protein nitrogen into protein will take place (106).

Several reports have shown that nitrogenous compounds affect the rate of cellulose digestion (11, 27, 28, 30, 57). The addition of urea to a diet containing 0.136 per cent of nitrogen, increased the cellulose digestion from 17.8 per cent to 38.7 per cent (57). Burroughs and Gerlaugh (28) studied the effect of soybean oil meal upon corn cob and timothy hay digestion in a fattening type ration for steers. They found an average increase of about 15 per cent in dry matter digestion when soybean oil meal was added. In later work (30) the addition of skimmilk to a diet containing corn cobs fed to steers increased roughage digestion, but only when 4 pounds of starch in the diet were present. Similar results were obtained when casein was added to a diet low in protein (27).

Indications that urea was more effective than soybean, linseed, cottonseed and corn gluten meal in stimulating cellulose digestion has been shown by the *in vitro* technique used by Belasco (11).

Effect of antibiotics, hormones, and vitamins on rumen activity.--The wide use of antibiotics in some livestock rations has promoted interest in studies of the effect of antibiotics upon rumen function. These substances in certain cases and under determined conditions have increased the amount of cellulose digested, but as a rule a decrease has occurred. Wasserman *et al*. (104) reported that penicillin, streptomycin, and neomycin stimulated the cellulolytic activity of rumen microorganisms; chloromycetin adversely affected the activity. Chlortetracycline (Aureomycin) has been shown to depress the activity of rumen microorganisms and cellulose digestion (75, 76). Prescott (94) has shown that terramycin, chlotetracycline, bacitracin and penicillin reduced the utilization of non-protein nitrogen by the rumen bacteria.

Brooks *et al*. (20) observed that the addition of 10 to 20 mcg. of diethylstilbestrol to a 25 ml. artificial rumen increased the digestion of cellulose an average of 9 per cent.

Riboflavin, vitamin B_{12}, biotin, PABA, pyridoxine, folic acid and a mixture of B vitamins have been reported to increase cellulose digestion *in vitro* (13, 55).

The literature relating to other aspects of ruminant nutrition has been excellently reviewed by others. The reader is referred to papers published by Doetsch and Robinson (39), Edwards (41), Elsden and Phillipson (42), Goss (51), Hastings (59), Huffman (64), Marston (81) and Owen (90).

EXPERIMENTAL PROCEDURE

Two types of purified cellulose were fermented in an artificial rumen under controlled conditions to determine the effect of varying concentrations of certain minerals upon the extent of cellulose digestion. Samples of rumen contents were obtained from a fistulated steer and from animals slaughtered at a local packing house. Rumen contents were filtered to remove solid materials, and the filtrate mixed with cellulose and a nutrient solution for fermentation in the artificial rumen. The extent of cellulose digestion was determined by chemical analysis for cellulose before and after fermentation and by feeding trials with rats and chicks.

Preparation of Artificial Rumen Inoculum

Rumen contents were obtained from a fistulated, 2-year-old Jersey steer. With this steer the effect of timothy and pangolagrass hay on cellulose digestion was studied. The hay was fed once a day about 8:00 A.M. (sufficient for ad-libitum feeding). An interval of 10 days occurred between the change from the timothy to the pangolagrass hay before rumen contents were obtained again. In addition to the hay, the steer was fed daily 5 lbs. of a concentrate mixture composed of: 200 lbs. of cottonseed meal (41 per cent protein), 200 lbs. crimped oats, 200 lbs. dried citrus pulp, 400 lbs. ground snapped corn, 10 lbs. salt, and 5 lbs. defluorinated rock phosphate. Fresh water and additional defluorinated rock phosphate and salt were available to the animal. A plastic plug described by Hentschel *et al*. (61) was used in the rumen fistula. When securing the rumen samples the contents were withdrawn in early afternoon, usually before the concentrate was fed.

Samples of rumen contents were also obtained from 24 mature beef type steers, slaughtered at a local abattoir. The rumen contents were removed within 5 to 10 minutes after the animals were slaughtered. These steers had been raised on pasture and received a variety of diets. The effect of these rations on cellulose digestion was compared to the studies with the fistulated steer.

The ingesta from all steers was strained through 4 layers of cheesecloth to remove the undigested solid materials. Three hundred milliliters of the filtrate were added to a 1 liter Erlenmeyer flask that contained 6 gm. of a purified cellulose, 100 ml. of distilled water and 200 ml. of a nutrient media recorded in Table 1. The nutrient media contained urea and many inorganic salts. As an element was investigated, it was deleted from this media. Two different sources of purified cellulose, known as Solka-Floc[1] and

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[1] Solka-Floc BW-40, Brown Co., Berlin, N. H.

TABLE 1

NUTRIENT SOLUTIONS USED IN THE ARTIFICIAL RUMEN

Constituent	Solution A[a]	Solution B[b]
	Gm./L	Gm./L
Urea	3.0	5.0
Na_2HPO_4	1.2	2.0
$NaHCO_3$	4.5	7.5
Na Cl	1.5	2.5
KH_2PO_4	1.5	2.5
$MgSO_4$ $7H_2O$	0.3	0.5
KCl	0.3	0.5
$CaCl_2$	0.3	0.5
$FeSO_4 \cdot 7H_2O$	0.150	0.249
$CuSO_4$	0.004	0.007
$CoCl_2 6H_2O$	0.006	0.010
$MnSO_4 \cdot H_2O$	0.009	0.015
$ZnSO_4 \cdot 7H_2O$	0.008	0.013

[a] Used in studying trace minerals. The element to be studied was not included in this solution.

[b] Nutrient solution used in the digestion of cellulose for monogastric animals.

Alphacel,[2] were used and compared in these studies. Before the rumen filtrate was added, the flask and other contents had been warmed in a constant temperature water bath at 40° C. for 15 minutes.

Immediately after the filtrate was added, carbon dioxide was bubbled into the mixture for 5 minutes. Aliquots were taken and pH determined. The pH of the mixture was adjusted to 7.2 with 4N Na_2CO_3 when necessary. The rumen inoculum was then ready to study the influence of various minerals on the digestion of cellulose.

Preparation of Nutrient Solutions

In Table 1 are shown 2 nutrient solutions containing urea and various salts. Solution A was used in the studies of the effect of cobalt, copper, iron, manganese, molybdenum and zinc upon artificial rumen digestion of cellulose. Solution B was used to study the efficiency of the artificial rumen in digesting cellulose for use by monogastric animals. In the use of nutrient solution A for the study of the particular element of interest, the element was deleted from the nutrient media, but added in various concentrations to the artificial rumen inoculum as described below. The elements studied were prepared in sufficient concentrations so that 1 ml. added to 24 ml. of rumen inoculum contributed the following concentrations of each element:

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[2] Nutritional Biochemicals Corporation, Cleveland 28, Ohio.

Cobalt ($CoSO_4 \cdot 7H_2O$): 0, 1, 5, 10, 25, 50, 100 and 250 p.p.m.

Copper ($CuSO_4 \cdot 5H_2O$): 0, 1, 5, 10, 20, 40, 60, 80 and 100 p.p.m.

Iron ($FeSO_4 \cdot 7H_2O$): 0, 5, 10, 50, 100, 250 and 500 p.p.m.

Manganese ($MnSO_4 \cdot H_2O$): 0, 1, 5, 10, 50, 100 and 250 p.p.m.

Molybdenum ($Na_2MoO_4 \cdot 2H_2O$): 0, 1, 5, 10, 25, 50, 100 and 250 p.p.m.

Zinc ($ZnSO_4 \cdot 7H_2O$): 0, 1, 5, 10, 25, 50, 100 and 250 p.p.m.

All the salts used were reagent grade.

An additional experiment was run using sodium sulphate (Na_2SO_4), at a concentration giving 500 p.p.m. of sodium and 1045 p.p.m. of sulfate in order to test the effect of the sulfate and sodium ions.

In Table 2, data are given for the concentration of the above elements that were found in the rumen filtrate of some steers used in these studies. All the samples were digested using per-chloric and nitric acid in the micro Kjeldahl apparatus. Copper and zinc were determined using zincon (83); cobalt using nitroso-R salt (96), iron using Ortho-phenanthroline (96), manganese using periodate (96) and molybdenum using thiocyanate-stannous chloride (96).

TABLE 2

CONCENTRATION OF ELEMENTS IN THE RUMEN FILTRATE OF STEERS FED PRACTICAL DIETS

Element	P.P.M.
Cobalt	0.06 ± 0.02[a]
Copper	0.36 ± 0.13
Iron	2.55 ± 0.51
Manganese	1.90 ± 0.41
Molybdenum	0.076 ± 0.02
Zinc	1.17 ± 0.39

[a] Mean values and standard deviation.

Determination of Effect of Minerals on Cellulose Digestion by Rumen Inoculum

To a 75 ml. Pyrex test tube was added 1 ml. of a solution that contained the particular concentration of the element being studied, and 24 ml. of the rumen inoculum containing the substrate, nutrient solution and rumen filtrate. These tubes were fitted with two-hole rubber stoppers and glass tubing used as an inlet and outlet for carbon dioxide. Carbon dioxide was continuously bubbled through the mixture to maintain anaerobic conditions and to provide agitation. Eighteen to 24 fermentation tubes were connected in series by the use of short pieces of rubber tubing. In all tests, two samples were retained for initial cellulose determination. The remaining tubes were incubated in a water bath at 40° C. for 24 hours, after which time the pH was determined and 1 ml. of 1 N H_2SO_4 added to stop fermentation. The digested samples were kept cold in a refrigerator at 38° F. until cellulose was determined, using the acetic-nitric acid procedure of Crampton and Maynard (37). This procedure was slightly modified and applied as follows: the samples, after digestion, were transferred to 50 ml. centrifuge tubes, and centrifuged for 1 minute at approximately 2,000 r.p.m. or 1,100 times gravity. The supernatant solution was decanted and 12 ml. of glacial acetic acid and 1.5 ml. of concentrated nitric acid were added to each residue. The tubes were then heated in a boiling water bath for 20 minutes and the contents filtered while hot through Gooch crucibles. The residue was transferred from the tubes and washed with 95 per cent ethyl alcohol, dried in an oven at 80° C., cooled in a dessicator and weighed, ashed in a muffle furnace at 675° C., cooled and weighed. The differences in weight between the acid-alcohol extracted dry residue and the ash were taken as the weight of cellulose present in the sample.

Use of Rats and Chicks to Determine the Extent of Cellulose Fermented in the Artificial Rumen

In this study, rumen contents were obtained only from the fistulated steer. The same procedure of preparing the rumen inoculum was followed as above except that larger quantities were used and nutrient solution B of Table 1 was used.

To a 6-liter Florence flask were added 200 grams of Alphacel, 1 liter of nutrient solution (Table 1), 3 liters of distilled water and the mixture warmed for 15 minutes in a water bath at 40° C. Then 1 liter of rumen filtrate was added, carbon dioxide bubbled through, pH adjusted to 7.2 with 4 N Na_2CO_3 if needed, and fermented for 24 hours. A mechanical stirrer was used throughout this period. The fermented material was placed in a shallow porcelain pan, dried at 80° C. for 36 hours and ground through a Wiley mill. This material was used in rat and chick diets to evaluate the extent of the artificial rumen digestion. Aliquot samples of the material were also analyzed for moisture, ash, crude fiber, crude protein, ether extract and nitrogen free extract. Results of the analyses are recorded in Table 3. The methods used in the analyses were those recommended by the Association of Official Agricultural Chemists (86).

TABLE 3

CHEMICAL ANALYSIS OF PURIFIED CELLULOSE (ALPHACEL) AND THE RESIDUE AFTER FERMENTATION IN THE ARTIFICIAL RUMEN

	Unfermented	Fermented
	(%)	(%)
Moisture	7.81 ± 0.73[a]	11.63 ± 0.82[a]
Ash	0.10 ± 0.02	8.42 ± 0.85
Crude Fiber	57.72 ± 1.92	49.96 ± 0.82
Crude Protein	0.00 ± 0.00	4.38 ± 0.90
Ether Extract	0.17 ± 0.09	0.55 ± 0.23
Nitrogen Free Extract	34.20 ± 2.52	25.06 ± 1.76

[a] Mean values and standard deviation.

Feeding trials with rats.--One hundred and twenty weanling rats of the Long-Evans strain, from the Nutrition Laboratory stock colony were used in these experiments. They were housed in groups of 3 or 4 in galvanized metal wire cages.

For the first trial, 60 rats, ranging in age from 23 to 25 days were randomized according to weight and sex into 3 treatments of 20 rats each (10 males and 10 females). They were fed the 3 diets of Table 4. The dietary groups received 4 per cent Alphacel (control), 15 per cent Alphacel (diet 2) and 15 per cent Alphacel fermented in the artificial rumen (diet 3).

The rats of diets 2 and 3 were fed the same weight of feed as eaten ad libitum by the control group. Fresh water was provided from glass watering bottles. Growth was determined from weekly weights made throughout the

TABLE 4

COMPOSITION OF DIETS FED TO RATS

Ingredient	Control	Diet 2	Diet 3
	(%)	(%)	(%)
Ground yellow corn	34.00	34.00	34.00
Dry whole milk	25.00	25.00	25.00
Soybean oil meal	12.00	12.00	12.00
Sucrose	16.00	5.00	5.00
Casein	6.70	6.70	6.70
Alfalfa leaf meal	1.50	1.50	1.50
NaCl	0.40	0.40	0.40
$CaCO_3$	0.40	0.40	0.40
Alphacel	4.00	15.00	----
Fermented Alphacel	-----	-----	15.00

TABLE 5

CHEMICAL ANALYSIS OF DIETS FED TO RATS

	Control	Diet 2	Diet 3
	(%)	(%)	(%)
Moisture	9.69	10.84	12.68
Ash	3.33	3.22	4.55
Crude Fiber	5.05	12.62	12.04
Crude Protein	19.83	19.59	20.55
Ether Extract	7.04	6.96	6.87
Nitrogen Free Extract	55.06	46.77	43.31

experimental period.

The experiment was repeated at a later time using the same number of rats and dietary treatments.

The results of chemical analysis (86) of the 3 diets for moisture, ash, crude fiber, crude protein, ether extract, and nitrogen free extract are recorded in Table 5.

Feeding trial with chicks.--One hundred and twenty unsexed chicks (Cornish x White Plymouth Rock corssbreed) one day old, were obtained from a local commercial hatchery at two different periods.

Initially, 60 chicks were randomized according to weight into 3 treatments of 20 chicks each. A commercial temperature-controlled battery was used to house each dietary group during the 4-week feeding period. The composition of the diets is given in Table 6. The 3 dietary groups received 15 per cent sucrose (control), 15 per cent Alphacel (diet 2) and 15 per cent Alphacel fermented in the artificial rumen (diet 3).

The diets were fed ad libitum and weekly records of feed consumption were taken. Fresh water was provided at all times. Growth was determined by weekly weights.

The experiment was repeated at a later time using the same number of chicks and dietary treatments.

The results of chemical analysis of the 3 diets for moisture, ash, crude fiber, crude protein, ether extract and nitrogen free extract are recorded in Table 7.

TABLE 6

COMPOSITION OF DIETS FED TO CHICKS

Ingredients	Control	Diet 2	Diet 3
	(%)	(%)	(%)
Ground yellow corn	33.00	33.00	33.00
Soybean oil meal	39.00	39.00	39.00
Alfalfa leaf meal	3.00	3.00	3.00
Fish meal	3.00	3.00	3.00
Dried whey	3.00	3.00	3.00
Steamed bone meal	1.50	1.50	1.50
Oyster shell flour	1.50	1.50	1.50
Salt	.50	.50	.50
Sucrose	15.00	-----	-----
Alphacel	-----	15.00	-----
Fermented Alphacel	-----	-----	15.00

Dietary Supplement	Per 100 Pounds of Feed
Vitamin A	14.00 gms.
Vitamin D	9.00 "
Calcium Pantothenate	1.00 "
Riboflavin	0.15 "
Niacin	1.50 "
Choline Chloride	12.70 "
Aurofac 2 A	57.00 "
Manganese Sulfate	5.70 "
Methionine	23.00 "

TABLE 7

CHEMICAL ANALYSIS OF DIETS FED TO CHICKS

	Control	Diet 2	Diet 3
	(%)	(%)	(%)
Moisture	8.59	9.98	12.60
Ash	6.92	6.78	9.22
Crude Fiber	4.84	12.47	9.46
Crude Protein	23.21	22.77	23.62
Ether Extract	1.41	0.98	1.18
Nitrogen Free Extract	55.03	47.02	43.92

Statistical Analysis

Statistical treatment of data followed the methods of Snedecor (98), Federer (43) and Cockran and Cox (36). The data were evaluated for significance by analysis of variance (F test) and by Duncan's multiple range test (43). This range test is designed to determine which values obtained in the various treatments are significantly different from one another.

RESULTS AND DISCUSSION

Effect of Trace Minerals on Cellulose Digestion Using the Artificial Rumen

In Tables 8 through 25 the data and statistical analysis summaries are presented that were obtained in the study of the influence of various levels of cobalt, copper, iron, manganese, molybdenum, and zinc on the digestion of cellulose by microorganisms in the artificial rumen. Two forms of purified cellulose, Solka-Floc and Alphacel, were used in each mineral study. Rumen filtrate from animals consuming timothy hay, pangolagrass hay and pasture was used in the study of each element and in the study of the two types of cellulose. The values tabulated are the means of four to six observations in each case. The data were evaluated for significance by analysis of variance (F test) and by Duncan's multiple range test. When comparing some of the results obtained with *in vivo* work, the assumption was made that an animal weighing 1,000 lbs. has a rumen capacity of 20 gallons.

The results recorded in these tables represent changes caused by the addition of certain trace minerals. The trace elements, present in the rumen inoculum (Table 2), were transferred with the filtrate to the fermentation mixture. Thus, the rumen inoculum contained small amounts of trace minerals contributed by the rumen filtrate plus additional known amounts of the element being studied.

Cobalt.--In Tables 8, 9, and 10 are presented the data obtained in the study of cobalt. The average percentages of cellulose digested when 0, 1, 5, 10, 25, 50, 100, and 250 p.p.m. of cobalt were added, were approximately 79, 79, 73, 68, 46, 28, 16, and 6 respectively. The addition of 1 p.p.m. of cobalt had no influence; but as increasing levels of the element were added, each addition resulted in a significant depression (P less than 0.01), in the amount of the cellulose digested.

Salsbury et al. (95) who worked with Solka-Floc as the source of cellulose and a dialyzing membrane-type artificial rumen found that 12 p.p.m. of cobalt depressed, and 100 p.p.m. of cobalt significantly decreased cellulose digestion. McNaught et al. (85) reported that 10 and 100 p.p.m. of cobalt in an artificial rumen resulted in a slight and tremendous decrease respectively in the utilization of non-protein nitrogen. In feeding experiments Becker et al (9), observed no toxic effects when cattle received 0.5 grams of cobalt per 1,000 lbs. of body weight daily. This amount of cobalt, if given at one time, would give a maximum concentration of approximately 6 p.p.m. in the rumen, calculated for a 1,000-lb. animal having a rumen capacity

TABLE 8

EFFECT OF COBALT UPON DIGESTION OF TWO TYPES OF PURIFIED CELLULOSE USING THE ARTIFICIAL RUMEN AND INOCULUM FROM ANIMALS FED DIFFERENT FORAGES

Cobalt added, p.p.m.	Percentage of Cellulose Digested					
	Timothy[a]		Pangola[a]		Pasture[a]	
	S.F.[b]	A.[c]	S.F.[b]	A.[c]	S.F.[b]	A.[c]
0	81.8	76.8	77.9	79.2	76.0	81.4
1	81.3	76.9	78.2	79.0	75.9	81.0
5	74.1	72.7	73.6	72.8	72.9	74.4
10	71.8	65.2	66.9	68.9	65.7	72.3
25	48.9	45.3	45.3	46.0	41.6	51.1
50	31.1	28.8	25.8	28.2	23.3	29.0
100	17.2	14.8	15.6	16.1	12.8	20.8
250	7.1	6.1	6.9	4.9	3.8	7.0

[a] Source of inoculum.

[b] Substrate = 1 per cent Solka-Floc.

[c] Substrate = 1 per cent Alphacel.

TABLE 9

SUMMARY OF ANALYSIS OF VARIANCE OF DATA IN TABLE 8

Source of Variation	DF	MS
Total	47	
Substrates	1	11.22
Inoculum	2	7.20
Error (a)	2	80.45
Levels	7	5,258.36**
Source x Levels	14	0.98
Error (b)	21	2.14

**Highly significant, P less than 0.01.

TABLE 10

RESULTS OBTAINED WITH DIFFERENT LEVELS OF COBALT USING THE DUNCAN'S MULTIPLE RANGE TEST[a]

Levels, added p.p.m.	250	100	50	25	10	5	1	0
Means, per cent Cellulose digested	5.96	16.21	27.70	46.36	68.46	73.41	78.71	78.85

[a] Any two means not underscored by the same line are significantly different (P less than 0.01). Any two means underscored by the same line are not significantly different.

of 20 gallons. Cornell workers (7, 8), in a series of studies investigating blood changes in sheep, found that as much as 0.16 gm. of cobalt per 100 lbs. of body weight daily, caused severely depressed appetite and losses in body weight.

From the results of this investigation, the probability of depressing normal bacterial action in the rumen by therapeutic treatment with quite large doses of cobalt seems very remote.

Copper.--The data obtained in the study of this element are presented in Tables 11, 12, and 13. The average percentages of cellulose digested when 0, 1, 5, 10, 20, 40, 60, 80, and 100 p.p.m. of copper were added, were approximately 78, 78, 71, 58, 17, 9, 7, 6, and 5 respectively. The addition of 1 p.p.m. of copper had no influence, but with increasing levels of copper the cellulose digested decreased until at 20 p.p.m., only about 22 per cent as much cellulose was digested as at 0 or 1 p.p.m. of added copper. These data are similar to those obtained by McNaught *et al.* (85), who reported that 10 and 25 p.p.m. of copper in an artificial rumen resulted in a slight and tremendous decrease respectively in the utilization on non-protein nitrogen. Copper has been demonstrated to be toxic to cattle. Kidder (70) fed 1 gm. of anhydrous copper sulfate per 100 lbs. of body weight to a steer pasturing on Saint Augustine grass and it died after 122 days. Henson (60) fed two

TABLE 11

EFFECT OF COPPER UPON DIGESTION OF TWO TYPES OF PURIFIED CELLULOSE USING THE ARTIFICIAL RUMEN AND INOCULUM FROM ANIMALS FED DIFFERENT FORAGES

Copper added, p.p.m.	Percentage of Cellulose Digested					
	Timothy[a]		Pangola[a]		Pasture[a]	
	S.F.[b]	A.[c]	S.F.[b]	A.[c]	S.F.[b]	A.[c]
0	82.3	76.6	80.7	80.1	75.8	75.4
1	81.9	76.3	80.2	79.6	77.2	75.2
5	76.2	72.6	75.6	70.0	66.1	66.4
10	60.1	54.3	64.1	58.8	56.2	56.5
20	16.6	18.0	13.5	14.6	25.6	15.4
40	6.1	9.8	8.7	5.3	17.8	9.1
60	7.5	7.5	6.5	4.6	10.3	7.7
80	7.9	3.7	4.8	5.3	9.2	6.6
100	6.9	3.4	3.8	5.1	6.9	5.4

[a] Source of inoculum.

[b] Substrate = 1 per cent Solka-Floc.

[c] Substrate = 1 per cent Alphacel.

TABLE 12

SUMMARY OF ANALYSIS OF VARIANCE OF DATA IN TABLE 11

Source of Variation	DF	MS
Total	53	
Substrate	1	78.72
Inoculum	2	0.61
Error (a)	2	2.42
Levels	8	6,776.71**
Source x Level	16	16.12
Error (b)	24	5.56

**Highly significant, P less than 0.01.

TABLE 13

RESULTS OBTAINED WITH DIFFERENT LEVELS OF COPPER USING THE DUNCAN'S MULTIPLE RANGE TEST[a]

Levels, added p.p.m.	100	80	60	40	20	10	5	1	0
Means, per cent Cellulose digested	5.25	6.25	7.35	9.46	17.28	58.33	71.15	78.40	78.48

[a] Any two means not underscored by the same line are significantly different (P less than 0.01). Any two means underscored by the same line are not significantly different.

steers in dry lot an equivalent amount of anhydrous copper sulfate. One died after 91 days with characteristic symptoms similar to those observed by Kidder; the other steer failed to develop any toxic symptoms. A maximum concentration of approximately 45 p.p.m. of copper occurred in the rumen contents of these steers, assuming that they had a rumen volume of 20 gallons.

The *in vitro* studies reported in this investigation suggest that copper is toxic in that it decreases cellulose digestion. However, if copper was present in the rumen at levels comparable to those used in the artificial rumen, harmful effects other than an effect upon cellulose digestion may be expected.

Iron.--The data obtained in the study of the effect of iron on cellulose digestion are presented in Tables 14, 15, and 16. These data indicate that iron has very little effect on cellulose digestion; however, the statistical analysis shows that the observed decrease of approximately 5 per cent, at the levels of 100 to 500 p.p.m. of iron, was significant (P less than 0.01). McNaught *et al*. (85) reported that a level of 1,000 p.p.m. of iron in the artificial rumen significantly decreased non-protein nitrogen utilization. In practical feeding of iron salts to cattle no reports of toxicity symptoms have appeared in the literature.

TABLE 14

EFFECT OF IRON UPON DIGESTION OF TWO TYPES OF PURIFIED CELLULOSE USING THE ARTIFICIAL RUMEN AND INOCULUM FROM ANIMALS FED DIFFERENT FORAGES

Iron added, p.p.m.	Percentage of Cellulose Digested					
	Timothy[a]		Pangola[a]		Pasture[a]	
	S.F.[b]	A.[c]	S.F.[b]	A.[c]	S.F.[b]	A.[c]
0	75.1	79.1	79.9	79.2	76.2	76.8
5	74.6	79.4	79.8	79.0	76.9	76.5
10	75.5	80.2	80.0	78.5	75.7	77.2
50	74.8	79.6	80.6	78.8	75.7	76.1
100	71.5	78.4	75.1	74.4	72.0	71.1
250	69.6	75.5	74.2	73.3	72.4	69.9
500	70.4	76.5	73.9	73.7	70.4	69.6

[a] Source of inoculum.

[b] Substrate = 1 per cent Solka-Floc.

[c] Substrate = 1 per cent Alphacel.

TABLE 15

SUMMARY OF ANALYSIS OF VARIANCE OF DATA IN TABLE 14

Source of Variation	DF	MS
Total	41	
Substrate	1	19.34
Inoculum	2	34.48
Error (a)	2	41.47
Levels	6	41.20**
Source x Level	12	0.99
Error (b)	18	0.49

**Highly significant, P less than 0.01.

TABLE 16

RESULTS OBTAINED WITH DIFFERENT LEVELS OF IRON USING THE DUNCAN'S MULTIPLE RANGE TEST[a]

Levels, added p.p.m.	500	250	100	50	5	0	10
Means, percent Cellulose digested	72.41	72.48	73.75	77.60	77.70	77.71	77.85

[a] Any two means not underscored by the same line are significantly different (P less than 0.01). Any two means underscored by the same line are not significantly different.

Manganese.--In Tables 17, 18, and 19 the data are recorded for results of the study with manganese. These observations show that 1 p.p.m. of manganese increased cellulose digestion by 2 per cent and 100 p.p.m. decreased it by 2 per cent. These changes were statistically significant. In comparing the effect of manganese with the effect of similar concentrations of cobalt, copper and zinc, manganese has very little practical effect. This is the first report of the effect of manganese on the digestion of cellulose by the artificial rumen. No report of toxicity of manganese in cattle feeding has appeared in the literature.

Molybdenum.--The data obtained in the study of the effect of molybdenum on cellulose digestion by the artificial rumen are presented in Tables 20, 21, and 22. The average percentages of cellulose digestion with 0, 5, 10, 20, 40, 60, 80, and 100 p.p.m. of added molybdenum were approximately 77, 76, 75, 74, 71, 70, 68, and 66 respectively. The decreases observed at the 20 p.p.m. and greater concentrations of molybdenum were statistically significant. However, the effect of comparable concentrations of cobalt, copper and zinc were much greater. McNaught *et al*. (85) reported that, in the artificial rumen using the utilization of non-protein nitrogen as an index, rumen bacteria would tolerate from 100 to 1,000 p.p.m. of molybdenum, but the utilization of

TABLE 17

EFFECT OF MANGANESE UPON DIGESTION OF TWO TYPES OF PURIFIED CELLULOSE USING THE ARTIFICIAL RUMEN AND INOCULUM FROM ANIMALS FED DIFFERENT FORAGES

Manganese added p.p.m.	Percentage of Cellulose Digested					
	Timothy[a]		Pangola[a]		Pasture[a]	
	S.F.[b]	A.[c]	S.F.[b]	A.[c]	S.F.[b]	A.[c]
0	75.7	78.1	73.8	75.1	77.6	78.9
1	78.0	79.2	76.0	77.4	78.6	80.7
5	76.6	80.0	74.6	75.8	77.0	79.5
10	75.6	78.5	74.2	74.6	77.0	79.1
50	75.6	76.7	73.7	74.8	76.8	78.7
100	74.7	78.6	73.2	71.5	76.1	78.1
250	74.9	77.8	73.8	73.9	76.7	78.2

[a] Source of inoculum.

[b] Substrate = 1 per cent Solka-Floc.

[c] Substrate = 1 per cent Alphacel.

TABLE 18

SUMMARY OF ANALYSIS OF VARIANCE OF DATA IN TABLE 17

Source of Variation	DF	MS
Total	41	
Substrate	1	29.17
Inoculum	2	49.32
Error (a)	2	3.66
Levels	6	5.70**
Source x Level	12	0.44
Error (b)	18	0.41

**Highly significant, P less than 0.01.

TABLE 19

RESULTS OBTAINED WITH DIFFERENT LEVELS OF MANGANESE USING THE DUNCAN'S MULTIPLE RANGE TEST[a]

Levels, added p.p.m.	100	250	50	10	0	5	1
Means, per cent Cellulose digested	75.36	75.88	76.05	76.50	76.53	77.25	78.31

[a] Any two means not underscored by the same line are significantly different (P less than 0.01). Any two means underscored by the same line are not significantly different.

TABLE 20

EFFECT OF MOLYBDENUM UPON DIGESTION OF TWO TYPES OF PURIFIED CELLULOSE USING THE ARTIFICIAL RUMEN AND INOCULUM FROM ANIMALS FED DIFFERENT FORAGES

Molybdenum added p.p.m.	Percentage of Cellulose Digested					
	Timothy[a]		Pangola[a]		Pasture[a]	
	S.F.[b]	A.[c]	S.F.[b]	A.[c]	S.F.[b]	A.[c]
0	69.3	71.5	79.9	77.0	81.6	80.9
5	68.4	70.8	79.2	76.8	80.7	79.5
10	67.5	70.1	79.2	75.7	81.2	79.4
20	63.4	67.7	78.0	75.9	78.5	78.0
40	61.8	65.0	76.3	74.5	75.7	75.2
60	60.3	62.9	75.4	71.2	75.1	72.7
80	58.9	60.4	73.5	71.6	72.4	71.3
100	56.7	58.5	74.4	68.1	70.1	66.1

[a] Source of inoculum.

[b] Substrate = 1 per cent Solka-Floc.

[c] Substrate = 1 per cent Alphacel.

TABLE 21

SUMMARY OF ANALYSIS OF VARIANCE OF DATA IN TABLE 20

Source of Variation	DF	MS
Total	47	
Substrate	1	5.81
Inoculum	2	672.67*
Pangola vs. Pasture	1	4.28
2 (Timothy) vs. Pangola and Pasture	1	1340.26*
Error (a)	2	34.69
Levels	7	98.09**
Source x Level	14	3.18**
Error (b)	21	0.75

*Significant, P less than 0.05.

**Highly significant, P less than 0.01.

TABLE 22

RESULTS OBTAINED WITH DIFFERENT LEVELS OF MOLYBDENUM USING THE DUNCAN'S MULTIPLE RANGE TEST[a]

Levels, added p.p.m.	100	80	60	40	20	10	5	0
Means, per cent Cellulose digested	65.65	68.01	69.60	71.41	73.58	75.51	75.90	76.70

[a] Any two means not underscored by the same line are significantly different (P less than 0.01). Any two means underscored by the same line are not significantly different.

non-protein nitrogen was significantly decreased at a level of 2,000 p.p.m. of molybdenum.

The results presented for molybdenum suggest that the "teartness" disease caused by a molybdenum toxicity is not caused by an interference with the activity of the rumen bacteria. The disease occurs in cattle grazing pastures, in which the molybdenum content of the forage is 100 p.p.m. or less in the dry matter. This would be expected to give a concentration of less than 10 p.p.m. in the rumen contents.

Zinc.--Data in Tables 23, 24, and 25 represent results of trials using various levels of zinc in the study of cellulose digestion by the artificial rumen. The average percentages of cellulose digested when the zinc concentrations were 0, 1, 5, 10, 25, 50, 100, and 250 p.p.m. were approximately 79, 79, 79, 75, 60, 32, 7, and 4 per cent respectively. The addition of 1 and 5 p.p.m. of zinc had no influence,but the 50 p.p.m. level decreased the digestion to approximately 41 per cent of that obtained when no zinc was added. At 100 p.p.m. only about 10 per cent of the normal fermentation took place.

This is the first report of the effect of zinc on the digestion of cellulose by the artificial rumen. Skipper (97) fed a concentrate containing 2,000 p.p.m. of zinc to young dairy calves from one through 27 weeks of age and observed no influence on growth or in several blood

TABLE 23

EFFECT OF ZINC UPON DIGESTION OF TWO TYPES OF PURIFIED CELLULOSE USING THE ARTIFICIAL RUMEN AND INOCULUM FROM ANIMALS FED DIFFERENT FORAGES

Zinc added p.p.m.	Percentage of Cellulose Digested					
	Timothy[a]		Pangola[a]		Pasture[a]	
	S.F.[b]	A.[c]	S.F.[b]	A.[c]	S.F.[b]	A.[c]
0	76.8	82.5	78.1	80.4	78.4	80.8
1	78.2	82.5	78.9	79.5	77.3	79.3
5	79.2	78.7	78.3	80.1	78.1	78.9
10	74.6	78.4	76.5	75.2	73.5	75.0
25	60.5	65.9	58.3	61.8	54.8	59.2
50	44.2	44.5	35.2	25.3	23.7	22.4
100	7.7	7.6	8.2	8.1	5.2	5.8
250	5.8	4.2	4.8	5.1	2.5	3.6

[a] Source of inoculum.

[b] Substrate = 1 per cent Solka-Floc.

[c] Substrate = 1 per cent Alphacel.

TABLE 24

SUMMARY OF ANALYSIS OF VARIANCE OF DATA IN TABLE 23

Source of Variation	DF	MS
Total	47	
Substrate	1	14.08
Inoculum	2	62.82
Error (a)	2	6.69
Levels	7	1,479.5 **
Source x Level	14	6.12
Error (b)	21	4.35

**Highly significant, P less than 0.01.

TABLE 25

RESULTS OBTAINED WITH DIFFERENT LEVELS OF ZINC USING THE DUNCAN'S MULTIPLE RANGE TEST[a]

Levels, added p.p.m.	250	100	50	25	10	5	1	0
Means, per cent Cellulose digested	4.33	7.10	32.5	60.0	75.5	78.8	79.2	79.5

[a] Any two means not underscored by the same line are significantly different (P less than 0.01). Any two means underscored by the same line are not significantly different.

constituents. The daily intake of the concentrate was increased from 1 to 4 lbs. during the experimental period. Possibly, the reason for the absence of any ill effects was that the rumen had not yet developed at the beginning of the feeding program and the bacteria may have become adjusted to the zinc by the time the rumen developed. However, the level of zinc fed should have given a concentration of zinc in the range of 100 p.p.m., which is very high.

Sodium sulfate.--Since the cobalt, copper, iron, manganese, molydenum and zinc ions were added in the above artificial rumen studies in the form of the sulfate or sodium salts, it was considered necessary to evaluate sodium and sulfate ions for possible effects. To do this, tests were made in which 500 p.p.m. of sodium and 1,045 p.p.m. sulfate ions as Na_2SO_4 were added to each articial rumen digestion tube. Eight determinations were made on both the purified Solka-Floc and Alphacel cellulose, using the timothy, pangola and pasture rumen inoculum. These high levels of sodium and sulfate ions had no effect on the extent of cellulose digestion. Therefore, the effect of cobalt, copper, iron, manganese, molybdenum and zinc, were due to the elements themselves.

Effect of type of cellulose and source of rumen inoculum upon cellulose digestion. The Solka-Floc and Alphacel were digested to the same extent in the presence of the various minerals and rumen filtrates. The filtrates from the 3 different sources of forage were not essentially different in their effect upon cellulose digestion. In one case, Table 20, filtrate from the period of timothy hay consumption caused the digestion of less cellulose than the other sources of rumen filtrate. The reason for the decreased digestion in this trial is unknown.

At the end of each digestion period the pH values were recorded and observed to fall in the range of 6.0 to 6.5. These figures are all within the normal range existing in the rumen, pH 6.0 to 7.2.

Feeding Value for Rats and Chicks of Cellulose Following Fermentation in the Artificial Rumen

Results of the feeding trials designed to study the feeding value for rats and chicks of cellulose following fermentation are recorded in Tables 26 through 30.

A test of the feeding value of fermented cellulose appeared desirable because of the potential application of this procedure in improving rations high in crude fiber for monogastric animals.

A statistical analysis of the growth data indicated that fermentation of cellulose in the artificial rumen had no beneficial influence on the rate of growth of the rats and chicks. However, in all cases with male and female rats, as well as the chicks, the fermented cellulose produced

TABLE 26

EFFECT OF CELLULOSE FERMENTED IN THE ARTIFICIAL RUMEN UPON THE GROWTH RATE OF MALE RATS

Weeks on Diet	Weights in Grams		
	Control	Diet 2[a]	Diet 3[b]
	Trial I		
	(10 rats)	(10 rats)	(10 rats)
Initial	48.2	51.9	50.9
1	75.7	76.9	79.4
2	108.9	105.5	108.8
3	138.1	129.8	135.5
4	181.1	164.3	169.0
5	205.1	176.8	183.5
6	232.5	199.4	205.9
Total gain, 6 wks.	184.3	147.5	155.0
	Trial II		
Initial	57.0	60.4	58.7
1	95.7	91.0	88.5
2	127.2	111.3	111.8
3	160.1	141.5	141.2
4	206.2	182.0	177.6
5	235.3	200.1	206.7
6	259.2	229.5	229.9
Total gain, 6 wks.	202.2	169.1	171.2

[a] 15 per cent unfermented Alphacel.

[b] 15 per cent fermented Alphacel.

TABLE 27

EFFECT OF CELLULOSE FERMENTED IN THE ARTIFICIAL RUMEN UPON THE GROWTH RATE OF FEMALE RATS

Weeks on Diet	Weights in Grams		
	Control	Diet 2[a]	Diet 3[b]
Trial I			
	(10 rats)	(10 rats)	(10 rats)
Initial	45.8	47.9	45.9
1	69.2	70.5	69.3
2	97.3	93.5	95.2
3	120.6	115.3	121.9
4	144.6	132.0	141.6
5	157.2	139.9	149.3
6	171.5	150.6	156.6
Total gain, 6 wks.	125.7	102.7	110.7
Trial II			
Initial	54.7	55.5	54.5
1	87.2	81.5	82.2
2	113.9	105.7	105.7
3	136.2	126.4	125.6
4	159.9	143.7	144.1
5	174.5	156.0	158.9
6	188.1	168.5	169.5
Total gain, 6 wks.	133.4	113.0	115.0

[a] 15 per cent unfermented Alphacel.

[b] 15 per cent fermented Alphacel.

TABLE 28

EFFECT OF CELLULOSE FERMENTED IN THE ARTIFICIAL RUMEN UPON THE GROWTH RATE OF CHICKS

Weeks on Diet	Weights in Grams		
	Control	Diet 2[a]	Diet 3[b]
Trial I			
	(20 chicks)	(20 chicks)	(17 chicks)
Initial	39.3	40.1	38.9
1	86.0	78.1	86.4
2	159.2	137.3	157.4
3	242.4	193.1	212.8
4	383.7	327.2	340.3
Total gain, 4 wks.	344.4	287.1	301.4
Trial II			
	(18 chicks)	(18 chicks)	(20 chicks)
Initial	36.6	38.7	39.0
1	75.1	71.9	74.0
2	145.3	127.5	135.9
3	248.6	209.5	227.7
4	386.7	318.2	335.1
Total gain, 4 wks.	350.1	279.5	296.1

[a] 15 per cent unfermented Alphacel.

[b] 15 per cent fermented Alphacel.

TABLE 29

SUMMARY OF ANALYSIS OF VARIANCE OF THE GROWTH DATA OF RATS

Source of Variation	DF	MS
Total	119	
Replications	1	5,070 **
Diets	2	9,166.95**
Sex	1	90,091.2 **
Control Diet vs. Diets 2 & 3	1	17,853.75**
Diet 2 vs. Diet 3	1	480.21
Sex x Diets	2	3,677.4 **
Error	113	186.66

**Highly significant, P less than 0.01.

TABLE 30

SUMMARY OF ANALYSIS OF VARIANCE OF THE GROWTH DATA OF CHICKS

Source of Variation	DF	MS
Total	112	
Replications	1	298.9
Subgroups	5	20,094.10**
Diets	2	42,073.44**
Control Diet vs. Diets 2 & 3	1	79,797.09**
Diet 2 vs. Diet 3	1	4,349.79
Replications x Diets	2	8,012.37
Error	107	2,971.37

**Highly significant, P less than 0.01.

better gains. The chicks responded slightly better than the rats. The growth of the rats and chicks consuming the control diets was significantly greater (P less than 0.01) than growth of rats and chicks consuming the experimental diets. The increased growth of chicks and rats consuming the control diet probably resulted from the greater proportion of available energy in the control diets.

The appetites and health of both the chicks and rats appeared normal throughout the feeding periods. No mortality occurred in the rats during the 2 six-week experimental periods. In Table 28 it is indicated that 7 chicks died during the tests. In all cases they died within the first 3 days of feeding and it appeared that death was due to causes other than the diets.

Muhrer et al. (89) fed a ruminant type ration, following fermentation in an artificial rumen, to a limited number of pigs. The results indicated that such a ration could be improved by fermenting some ingredients of the ration. Comparing these results with the data above, it is indicated that the artificial rumen could be used to improve diets high in cellulose for monogastric animals, but modification of the technique is necessary to make possible more satisfactory results. Such modification could involve a longer fermentation period, increase or decrease the amount of rumen filtrate, better control of the pH, and possibly other factors.

The pH values recorded after the 24-hour fermentation periods were in the range of 5.3 and 5.7, and probably within this range the low pH was harmful to the cellulolytic activity of the rumen bacteria.

SUMMARY

An artificial rumen was used to study the effect of cobalt, copper, iron, manganese, molybdenum and zinc on the digestion of two kinds of purified cellulose in the presence of rumen inoculum from animals consuming different roughages. The amount of cellulose digested was determined chemically and the feeding value of fermented cellulose was determined by feeding trials with rats and chicks. Conclusions which may be made from the experiments are summarized in the following statements.

1. The addition of 1 p.p.m. of cobalt had no influence upon the amount of cellulose digested, but as increasing levels of the element were added, each addition resulted in a significant depression. Ten p.p.m. slightly depressed and 100 p.p.m. strongly depressed cellulose digestion.

2. One p.p.m. of copper added to the substrate and reaction medium in the artificial rumen had no influence in the amount of cellulose digested, but with increasing levels the cellulose digested decreased greatly, until at 20 p.p.m., only 22 per cent as much cellulose was digested as at 0 or 1 p.p.m. of added copper.

3. The addition of 1 and 5 p.p.m. of zinc had no influence upon the amount of cellulose digested, but the 50 p.p.m. level decreased the digestion to about 41 per cent of that obtained when no zinc was added. At 100 p.p.m. only about 10 per cent of the normal fermentation took place.

4. Twenty p.p.m. and greater concentrations of molybdenum significantly decreased cellulose digestion. Levels of 5 and 10 p.p.m. of molybdenum had no influence.

5. The addition of iron up to 500 p.p.m. and manganese up to 250 p.p.m. slightly decreased cellulose digestion, but the decrease was much less than that caused by above elements.

6. The addition of sodium and sulfate ions had no influence upon the amount of cellulose digested.

7. There was no difference in the amount of cellulose digested when Solka-Floc or Alphacel were used as substrates. Three different sources of rumen inoculum did not affect the rate of cellulose digestion.

8. No significant beneficial effects were obtained as measured by growth when rats and chicks were fed a purified source of cellulose fermented in an artificial rumen for 24 hours.

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BIOGRAPHICAL SKETCH

Juan Carlos Sala was born March 27, 1930, at Lima, Peru. He was graduated from La Salle School of Lima, Peru, in December, 1946. He entered the Escuela Nacional de Agricultura of Lima in April, 1948. In November, 1950, he received a scholarship from the University of Florida, and transferred to this school in February 1951, obtaining the degree of Bachelor of Science in Agriculture with high honors in February, 1952. He received the Master of Science degree in Agriculture in August, 1953, from the University of Florida.

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This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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